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(54) Title: GENETIC ALTERATIONS THAT CORRELATE WITH LUNG CARCINOMAS

(57) Abstract

The present invention relates to compositions and methods for detecting chromosome abnormalities correlated with lung cancer. The method contacting a nucleic acid sample from a patient with a probe which binds selectively to a target polynucleotide sequence correlated with lung cancer.

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GENETIC ALTERATIONS THAT CORRELATE WITH
LUNG CARCINOMAS

5   BACKGROUND OF THE INVENTION

The present invention provides methods for
detecting DNA copy number changes associated with
neoplastic growth. In particular, it provides methods
and compositions for the localization of chromosomal
10   regions of amplification or deletion associated with lung
cancers.

Over 140,000 new cases of lung cancer are
diagnosed each year, making lung cancer the most common
cause of cancer-related death in the United States
15   (Johnson and Greco, *Crit. Rev. Onc. Hemat.* 4:303-336
(1986)). One fourth of lung cancer cases are classified
as "small cell lung carcinoma" (SCLC), which is
distinguished from a variety of cancers referred to as
"non-small cell lung cancer" (N-SCLC) based on histology,
20   biochemical markers and clinical behavior. Major
treatment decisions are made on the basis of the
histological classification of a tumor as SCLC or N-SCLC.

The majority of patients with SCLC have
metastases at the time their cancer is diagnosed.
25   Despite the fact that SCLC initially shows great
sensitivity to radiation therapy and chemotherapy, most
patients relapse and die from their tumors within two
years of diagnosis (Sieffer and Ihde, *Seminars in
Oncology* 15:278-299 (1988)). Although the introduction
30   of combination chemotherapy and radiotherapy to the
treatment of SCLC has greatly improved the average
survival time of patients with this disease, the
prognosis is still very poor (Viallet and Ihde, *Crit.
Rev. Onc. Hemat.* 11:109-135 (1991)).

35   In contrast, N-SCLC are typically found to be
localized at the time of presentation and are generally
considered for either surgery or radiotherapy. The

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response of N-SCLC to chemotherapy usually is not
dramatic. This therapy is less important in metastatic
disease than it is in SCLC.

Increased understanding of the genetics of
5 tumorigenesis and response to radiation of lung cancers
will lead to better techniques of early diagnosis and for
the prediction of response to radiotherapy. For example,
improved techniques for the early diagnosis of SCLC based
on detection of early genetic aberrations may allow
10 earlier treatment (Birrer and Brown *Cancer Res. (Suppl.)*
52:2658s-2664s (1992)) before metastasis has occurred.
Identification of genetic aberrations that correlate with
radioresponse may lead to an assay that will allow
stratification of patients into groups that will benefit
15 from more (or less) aggressive radiotherapy or that will
be candidates for other therapeutic modalities.

Several genetic alterations have been
identified in both SCLC and N-SCLC tumors, including
changes in known oncogenes and tumor suppressor genes
20 (*see, e.g.,* Birrer and Brown, *supra*) aneusomies,
translocations, regional chromosomal deletions,
heterogeneously staining regions and double minute (DM)
chromosomes (Wurster-Hill *et al., Cancer Gen. Cytogen*
13:303-330 (1984); Ibson *et al., J. Cell. Biochem*
25 33:267-288 (1987)); Morstyn *et al., Cancer Res.* 47:3322-
3327 (1987)). With the exception of RB, a putative tumor
suppressor on 3p, and possibly p53, none of the
alterations identified so far are good candidates for the
earliest events in the progression of SCLC and none
30 correlate well with tumor response to radiotherapy.

The detection of amplified or deleted
chromosomal regions has traditionally been done by
cytogenetics. In complex karyotypes with multiple
translocations and other genetic changes, traditional
35 cytogenetic analysis is of little utility because
karyotype information is lacking, or cannot be
interpreted. Teyssier, J.R., *Cancer Genet. Cytogenet.*

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37:103 (1989). Furthermore, conventional cytogenetic banding analysis is time consuming, labor intensive, and frequently difficult or impossible.

Comparative genomic hybridization (CGH) is a more recent approach to identify the presence and localization of amplified or deleted sequences. *See*, Kallioniemi, *et al.* *Science* 258:818 (1992) and WO 93/18186. CGH reveals amplifications and deletions irrespective of genome rearrangement. CGH can provide a quantitative estimate of DNA copy number and also provides information regarding the localization of amplified or deleted sequences in the normal chromosome.

The use of CGH and related techniques to identify the genetic events leading to neoplastic transformation involved in lung cancers can facilitate efforts to define the biological basis for disease, improve prognostication and prediction of therapeutic response, and permit earlier tumor detection. The present invention addresses these and other needs.

SUMMARY OF THE INVENTION

The present invention provides compositions and methods of detecting a chromosome abnormality correlated with lung cancer. The methods comprise contacting a nucleic acid sample from a patient with a probe which binds selectively to a target polynucleotide sequence correlated with lung cancer. The probe is contacted with the sample under conditions in which the probe binds selectively with the target polynucleotide sequence to form a hybridization complex. The formation of the hybridization complex is then detected.

Alternatively, sample DNA from the patient can be flourescently labeled and competitively hybridized against florescently labeled normal DNA to normal lymphocyte metaphases. Alterations in DNA copy number in the sample DNA are then detected as increases or decreases in sample DNA as compared to normal DNA.

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The chromosome abnormality is typically a deletion or an amplification. The methods can be used to detect both small cell and non-small cell lung cancers.

5 Definitions

A "nucleic acid sample" as used herein refers to a sample comprising DNA in a form suitable for hybridization to a probes of the invention. For instance, the nucleic acid sample can be a tissue or cell

10 sample prepared for standard *in situ* hybridization methods described below. The sample is prepared such that individual chromosomes remain substantially intact and typically comprises metaphase spreads or interphase nuclei prepared according to standard techniques.

15 The sample may also be isolated nucleic acids immobilized on a solid surface (*e.g.*, nitrocellulose) for use in Southern or dot blot hybridizations and the like. In some cases, the nucleic acids may be amplified using standard techniques such as PCR, prior to the

20 hybridization. The sample is typically taken from a patient suspected of having a lung cancer associated with the abnormality being detected.

As used herein a "probe" is defined as a polynucleotide (either RNA or DNA) capable of binding to

25 a complementary target cellular genetic sequence through one or more types of chemical bonds, usually through hydrogen bond formation. It will be understood by one of skill in the art that probes will typically substantially bind target sequences lacking complete complementarity

30 with the probe sequence depending upon the stringency of the hybridization conditions. The probes are preferably directly labelled as with isotopes or indirectly labelled such as with biotin to which a streptavidin complex or fluroescently labeled antibody may later bind. By

35 assaying for the presence or absence of the probe, one can detect the presence or absence (or copy number) of

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the target. Nucleic acid probes can be prepared by a variety of methods known to those of skill in the art.

"Chromosome abnormalities" as used herein refers to any of several types well known to those of
5 skill in the art, including, but not limited to, extra or missing individual chromosomes, extra or missing portions of a chromosome (segmental duplications or deletions), breaks, rings and chromosomal rearrangements. Chromosomal rearrangements include translocations,
10 dicentrics, inversions, insertions, amplification and deletions.

A chromosome region or a target polynucleotide sequence is said to be correlated with lung cancer if deletion, amplification, or other rearrangement of the
15 region is found in a significant proportion (typically greater than about 30%, usually greater than about 50%) of lung cancer cell lines or tumors.

"Bind(s) substantially" refers to complementary hybridization between an oligonucleotide and a target
20 sequence and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired detection of the target polynucleotide sequence.

"Hybridizing" refers the binding of two single
25 stranded nucleic acids via complementary base pairing.

"Nucleic acid" refers to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, would encompass known analogs of natural nucleotides that can
30 function in a similar manner as naturally occurring nucleotides.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-E show sample green to red fluorescence ratio profiles generated by integration of
35 the fluorescence intensity along the axis of FITC-avidin (a green fluorochrome) and anti-digoxigenin rhodamine (a red fluorochrome) visualized target chromosome.

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Figure 2 is a chromosome schematic depicting the regions of chromosomal amplification and deletion observed for classic and variant SCLC cell lines.

Figure 3 is a chromosome schematic depicting
5 the gains and losses in non-small cell lung cancer cell lines.

Figure 4 is a chromosome schematic depicting the gains and losses in primary small cell lung cancers.

Figure 5 is a chromosome schematic depicting
10 the gains and losses in primary N-SCLC.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention is based on a comprehensive molecular cytogenetic analysis of the
15 genomes of SCLC and N-SCLC cell lines and tumors using comparative genetic hybridization (CGH, Kallioniemi et al., supra). This technique is a variation of a fluorescence in situ hybridization (FISH) technique which allows the simultaneous examination of the entire genome
20 of a given cell line or tumor for DNA copy number abnormalities in a single experiment.

The nucleotide sequences, typically DNA, used in the hybridization described below may be detectably labeled prior to the hybridization reaction.
25 Alternatively, a detectable label may be selected which binds to the hybridization product. The nucleotide sequences may be labeled with any detectable group for use in practicing the invention. Such detectable group can be any material having a detectable physical or
30 chemical property. Such detectable labels have been well-developed in the field of immunoassays and in general most any label useful in such methods can be applied to the present invention. Thus a label is any composition detectable by spectroscopic, photochemical,
35 biochemical, immunochemical, or chemical means. Useful labels in the present invention include fluorescent dyes, electron-dense reagents, enzymes (as commonly used in an

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ELISA), biotin, dioxigenin, or haptens and proteins for
which antisera or monoclonal antibodies are available.
The particular label used is not critical to the present
invention, so long as it does not interfere with the *in
situ* hybridization of the sequence.   In addition, the
label must be detectable in as low a copy number as
possible, thereby maximizing the sensitivity of the
assay, and yet be detectable above any background signal.
Finally, a label must be chosen that provides a highly
localized signal thereby providing a high degree of
spatial resolution.   In a preferred embodiment, the label
is digoxigenin-11-dUTP or biotin-14-dATP, which are then
detected using fluorescence excitation.

        The labels may be coupled to the DNA in a
variety of means known to those of skill in the art.   In
a preferred embodiment the probe will be labeled using
nick translation or random primer extension (Rigby, et
al. *J. Mol. Biol.*, 113: 237 (1977) or Sambrook, *et al.*,
Molecular Cloning - A Laboratory Manual, Cold Spring
Harbor Laboratory, Cold Spring Harbor, N.Y. (1985)).

        Standard *in situ* hybridization techniques are
then used to probe a given sample (typically a metaphase
spread).   Hybridization protocols for the particular
applications disclosed here are described in detail
below.   Several guides to the techniques are available,
e.g., Gall *et al.* *Meth. Enzymol.*, 21:470-480 (1981) and
Angerer *et al.* in *Genetic Engineering: Principles and
Methods* Setlow and Hollaender, Eds. Vol 7, pgs 43-65
(plenum Press, New York 1985).

        Briefly, a chromosomal sample is prepared by
depositing cells, either as single cell suspensions or as
tissue preparation, on solid supports such as glass
slides and fixed by choosing a fixative which provides
the best spatial resolution of the cells and the optimal
hybridization efficiency.

        Generally, *in situ* hybridization comprises the
following major steps: (1) fixation of tissue or

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biological structure to be analyzed; (2) prehybridization
treatment of the biological structure to increase
accessibility of target DNA, and to reduce nonspecific
binding; (3) hybridization of the mixture of nucleic
5   acids to the nucleic acid in the biological structure or
tissue; (4) posthybridization washes to remove nucleic
acid fragments not bound in the hybridization and (5)
detection of the hybridized nucleic acid fragments. The
reagent used in each of these steps and their conditions
10   for use vary depending on the particular application.
Hybridization protocols for the particular applications
disclosed here are described in detail below and in
Pinkel et al. Proc. Natl. Acad. Sci. USA, 85:9138-9142
(1988), WO 93/18186 and EPO Pub. No. 430,402.

15   In some applications it is necessary to block
the hybridization capacity of repetitive sequences. In
this case, human genomic DNA is used as an agent to block
such hybridization. The preferred size range is from
about 200 bp to about 1000 bases, more preferably between
20   about 400 to about 800 bp for double stranded, nick
translated nucleic acids.

              The FISH methods for detecting chromosomal
abnormalities described herein can be performed on
nanogram quantities of the subject nucleic acids.
25   Paraffin embedded tumor sections can be used, as can
fresh or frozen material. Because FISH and CGH can be
applied to the limited material, touch preparations
prepared from uncultured primary tumors can also be used
(see, e.g., Kallioniemi, A. et al., Cytogenet. Cell
30   Genet. 60: 190-193 (1992)). For instance, small biopsy
tissue samples from tumors can be used for touch
preparations (see, e.g., Kallioniemi, A. et al.,
Cytogenet. Cell Genet. 60: 190-193 (1992)). Small
numbers of cells obtained from aspiration biopsy or cells
35   in bodily fluids (e.g., blood, urine, sputum and the
like) can also be analyzed. For detection of lung
cancers, sputum samples are particularly preferred.

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In CGH, test (tumor) DNA uniformly labeled with a green fluorochrome and normal DNA uniformly labeled with a red fluorochrome are simultaneously hybridized to normal human metaphase chromosomes. Excess unlabeled

5   repetitive DNA (e.g., Cot-1 DNA) included in the hybridization mixture inhibits hybridization of respective sequences that are interspersed throughout the genome or located at the chromosome centromeres. Increases and decreases in the green to red fluorescence

10   ratio along each chromosome indicate regions of increased or decreased copy number in the tumor relative to normal DNA, respectively.

CGH was used to analyze 18 SCLC cell lines and 10 tumors and 5 N-SCLC cell lines and 20 tumors to

15   identify regions of the genome that contain previously unrecognized oncogenes and tumor suppressor genes, and other genes that play a role in tumorigenesis and/or radioresistance. The SCLC lines are categorized as "classic" or "variant" based on their cellular

20   morphologies, growth phenotypes and enzymatic activities (Gazdar et al. Cancer Research 45:2924-2930 (1985)). Cell lines of the variant phenotype are also more resistant to killing by ionizing radiation than those of the classic phenotype (Carmichael, Eur. J. Cancer Clin.

25   Oncol. 25:527-534 (1989)). These studies show several DNA copy number aberrations that have been identified previously and several that are novel. Importantly, 4 regions of common aberration correlate with response to radiation.

30   Genomic regions that are found to be sites of increased DNA copy number in a large fraction of the cell lines are likely to include oncogenes that are present at increased copy number and hence overexpressed. Overexpression of these genes may lead to uncontrolled

35   growth. Regions that frequently show a decreased DNA copy number may contain tumor suppressor genes that through mutation of one allele and deletion on the other

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lead to loss of growth or organizational control
(Weinberg, *Science* 254:1138-1146 (1992)). Of course,
some of the DNA copy number abnormalities may arise as
secondary consequences of general genomic instability
5 resulting from the early stages of tumorigenesis. Such
alterations are expected to occur randomly and,
therefore, are not likely to be found in a high
percentage of tumors and cell lines.

CGH generally detects unit changes in relative
10 DNA content larger than about 10 Mb although smaller
regions of increased copy can be detected if the level of
increase is several-fold. In addition, CGH does not
detect point mutations, small deletions or amplifications
or genetic rearrangements, such as translocations. With
15 these limitations in mind, changes identified here are
referred to as DNA copy number increases and number
decreases, rather than amplifications and deletions.

Copy number changes that affect whole
chromosomes are most likely the result of aneuploidies.
20 It is not surprising that many such changes were found as
most of the cell lines used in this study are known to be
near triploid or tetraploid with a wide range of
chromosome numbers (Wurster-Hill *et al.* *Cancer Gen.*
Cytogen 13:303-330 (1984); Zech *et al.* *Cancer Gen.*
25 *Cytogen.* 15:335-347 (1985)). However, cell lines with a
perfect triploid or tetraploid genetic complement would
appear normal by CGH as the relative numbers of each DNA
sequence are the same as in a normal cell. A tetraploid
cell line missing two copies of a given sequence would
30 give the same CGH profile as a diploid cell line missing
one copy. In these instance, the absolute level of a
copy number change can be ascertained by using a
single-copy probe for the affected region to perform FISH
on the cell line in question, using the methods described
35 below. However, in cases where a localized bright green
band is observed on a chromosome arm and the fluorescence

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ratio profile shows a high-level peak, such alterations
are considered to be amplifications.

     The new regions of amplification or deletion
described below can be studied in more detail using
5   chromosome specific painting with a collection of probes
that span the amplified or deleted region using the FISH
techniques described above.   For instance, a selected
chromosome may be isolated by flow cytometry and then
digested with restriction enzymes appropriate to give DNA
10   sequences of at least about 20 kb and more preferably
about 40 kb.   Techniques of partial sequence digestion
are well known in the art.   See, for example Perbal, *A
Practical Guide to Molecular Cloning* 2nd Ed., Wiley N.Y.
(1988).   The resulting sequences are ligated with
15   vectors suitable for large DNA sequences such as cosmids,
yeast artificial chromosomes (YACs), bacterial artificial
chromosomes (BACs) and P1 phage.   In addition, libraries
spanning entire chromosomes are available commercially
(Clonetech, South San Francisco, CA) or from the Los
20   Alamos National Laboratory.

     Once a probe library is constructed, a subset
of the probes is physically mapped on the selected
chromosome or chromosome region defined below.   FISH and
digital image analysis can be used to localize cosmids
25   along the desired chromosome.   This method is described
in Lichter *et al.*, *Science*, 247:64-69 (1990).   Briefly,
the clones are mapped by FISH to metaphase spreads from
normal cells using *e.g.*, FITC as the fluorophore.   The
chromosomes are counterstained by a stain which stains
30   DNA irrespective of base composition (*e.g.*, propidium
iodide), to define the outlining of the chromosome.   The
stained   metaphases   are   imaged   in   a   fluorescence
microscope with a polychromatic beam-splitter to avoid
color-dependent image shifts.   The different color images
35   are acquired with a CCD camera and the digitized images
are stored in a computer.   A computer program is then
used to calculate the chromosome axis, project the two

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(for single copy sequences) FITC signals perpendicularly onto this axis, and calculate the average fractional length from a defined position, typically the p-telomere.

Once a region of interest has been mapped using
5 the probes, one of skill will recognize that there are numerous means of further defining and/or screening for this region. The region may be sequenced by digesting chromosomal DNA with restriction enzymes and identifying the specific duplication-bearing fragments using mapped
10 cosmids as hybridization probes. The positive clones may then be subcloned into appropriate vectors and sequenced.

Sequence information permits the design of highly specific hybridization probes or amplification primers suitable for detection of the target sequences.
15 This is useful for diagnostic screening systems as well as research purposes.

Means for detecting specific DNA sequences in a variety of diagnostic and other applications are well known to those of skill in the art. For instance,
20 oligonucleotide probes chosen to be complementary to a select subsequence with the region can be used. Alternatively, sequences or subsequences may be amplified by a variety of DNA amplification techniques (for example via polymerase chain reaction, ligase chain reaction,
25 transcription amplification, etc.) prior to detection using a probe. Amplification of DNA increases the sensitivity of the assay by providing more copies of possible target subsequences. In addition, by using labeled primers in the amplification process, the DNA
30 sequences may be labeled as they are amplified.

The present invention further provides kits for the detection of the chromosomal abnormalities identified here. The kits comprise a compartment which contains a nucleic acid probe which binds selectively to a target
35 polynucleotide sequence within these chromosomal aberrations. The probes may further comprise appropriate labels well known to those of skill in the art.

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EXAMPLES

The following examples are provided by way of illustration only and not by way of limitation. Those of skill will readily recognize a variety of noncritical
5   parameters which could be changed or modified to yield essentially similar results.

Example 1: Comparative Genomic Hybridization (CGH) Analysis of Small Cell Lung Carcinoma (SCLC) Cell Lines
10   In order to detect genetic amplifications and deletions in SCLC cell lines, CGH was performed on SCLC cell lines using DNA from normal human male lymphocytes for comparison. Excess unlabeled blocking DNA was used to insure specificity.

15

Methodology
Cell culture and DNA extraction: Small cell lung carcinoma cell lines were obtained from the ATCC. Cell lines were grown in RPMI 1640 medium supplemented with
20   10% fetal bovine serum and antibiotics.   DNA was extracted from cell lines by a proteinase K/SDS digestion followed by phenol/chloroform/isoamyl alcohol extractions either manually (Sambrook et al., 1989) or using the Applied Biosystems, Inc. DNA extractor model 340A.

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DNA labeling: Chromosomal DNA was labeled with biotin-14-deoxyadenosine   triphosphate   or digoxigenin-11-deoxyuridine   triphosphate   (Boehringer Mannheim) using the Gibco/BRL Bio-Nick kit.   Reactions
30   were performed for 1 hr. at 15°C according to the manufacturer's instructions, using a range of DNAse I/Pol I concentrations in order to generate DNA fragments ranging from 200 bp to 2.3 kb (optimal for CGH).

35   Comparative genomic hybridization: Hybridizations were performed essentially as in Kallioniemi *et al.*, *supra*. Normal human male lymphocyte metaphase preparations were

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denatured in 70% formamide and 2x SSC (1x SSC is 0.15 M NaCl and 0.015 Na citrate (pH 7)) at temperatures ranging from 72°C to 78°C for 2.5 to 10 minutes, depending on the age and quality of the slides. The slides were then
5   dehydrated in a sequence of 70%, 85% and 100% ethanol washes. The slides were air-dried and incubated at 37°C until the probes were applied (less than 5 minutes). One hundred and twenty nanograms of biotinylated SCLC cell line DNA, 120 ng digoxygenin-labeled normal reference DNA
10 and 5 μg of Cot-1 blocking DNA (Gibco/BRL) were precipitated with ethanol. The pellet was resuspended in 3 μl of dH₂O and mixed with 7 μl of denaturation buffer to give a final concentration of 50% formamide, 10% dextran sulfate and 2x SSC (pH 7). This probe mixture was
15 denatured for 5 minutes at 77°C and incubated at 37°C for several minutes before being applied to the metaphase spreads. Cover slips were applied and sealed to the slides with rubber cement, and the slides were incubated in a humidified chamber for 3 days at 37°C.
20   Slides were washed at 45°C in three changes of 50% formamide/2x SSC (pH 7), followed by two washes with 2x SSC and one of 0.1x SSC (10 minutes in each wash). All subsequent manipulations were at room temperature. The slides were washed for five minutes in 2x SSC and
25 blocked for 5 minutes in 2x SSC+1% BSA (Sigma Pentax Fraction V). The slides were then immunohistochemically stained with 5 μg/ml FITC-avidin (a green fluorochrome; Vector Laboratories, Burlingame, CA) and 2 μg/ml anti-digoxigenin rhodamine (a red fluorochrome;
30 Boehringer Mannheim) in 2x SSC+1% BSA for 30 minutes in the dark. The slides were then washed in the dark for 10 minutes successively in each of the following: 4x SSC, 4x SSC containing 0.1 % Triton X-100 (Fluka Chemika), 4x SSC and PN (0.1 M NaH₂PO₄, 0.1 M Na₂HPO₄ and 0.1% NP-40).
35 The slides were drained on a paper towel, and 7.5 μl of a solution of 0.1 μM 4,6-di-amino-2-phenylindole (DAPI)

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was applied.   Cover slips were applied and sealed with
nail polish.

Image Acquisition and Processing: The slides were
5   examined using a Zeiss Axioplan fluorescence microscope.
Metaphases were photographed and stored as three color
images   (DAPI, FITC and rhodamine) using a cooled
charge-coupled device camera (Photometrics, Tucson, AZ)
interfaced with a Sun 4/330 work station using the
10   Quantitative   Image   Processing   System   (QUIPS).
Chromosomes were identified based on the DAPI banding
patterns (Kallioniemi et al., 1992).   The green to red
fluorescence   ratio   profiles   were   determined   by
integration of the fluorescence intensity along the axis
15   of each chromosome (Kallioniemi et al, 1992).
                Precise assignment of copy number increases and
decreases to chromosomal bands was accomplished by
looking   for   green   or   red   chromosomal   regions,
respectively, and noting their X and Y coordinates.   The
20   DAPI bands corresponding to these regions were then
determined by finding the same coordinates in the DAPI
image.   The extent and location of each copy number
change was then plotted on an idiogram showing a
schematic representation of the chromosomes G-banding
25   pattern.

Comparison of CGH data
                Figure   1   shows   representative   CGH   data
illustrating DNA copy number abnormalities detected using
30   CGH.   Representative green to red fluorescence ratios
corresponding to the red and green digital images of
individual chromosomes are shown.   Panel A shows a major
region of increased copy number relative to normal DNA on
chromosome 8 for the SCLC cell line NCI-H510A at 8q21-22.
35   NCI-HS10A was not known to contain any alterations on
chromosome 8.   Thus, the centromere proximal copy number
increase at 8q21-22 represents a previously unrecognized

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copy number increase.   NCI-H510A cells also contain a
minor copy number increase at 8q24, near the map position
of the c-myc oncogene.   However, Southern hybridization
shows that this cell line does not contain a c-myc
5   amplification (Brooks et al. (1987) *Advances in Viral
Oncology* 7, 155-172).   Thus, the present example
demonstrates that NCIH-510A cells contain two copy number
increases on chromosome 8 which were previously unknown.
Panel B shows that NC-H510A cells have an increased DNA
10   copy number at chromosome 5p.   Copy number increases on
5p have not been previously reported to be a frequent
cytogenetic alteration in SCLC cells or tumors.   However,
we observed copy number increases on 5p in 11 of 18 cell
lines and in 8 of 10 tumors, making the increase at 5p
15   one of the most common cytogenetic alterations in SCLC
cells and tumors.   Panels C and D show copy number
decreases for the cell line NCI-H211.   Copy number
decrease at 3p (Figure 1, panel C) is known to be among
the most common genetic alterations in SCLC (Brauch et
20   al.(1987) *N. Engl. J. Med.* 317, 1109-1113; Carbone and
Minna (1992) *Advances in Internal Medicine* 37, 153-171.),
and it has been suggested that one or more tumor
suppressor loci may reside there.   Figure 1, panel D
shows a decrease at 4q22-35. Although this has not been
25   repeated previously, we observed decreases at this site
in over half (10/18) of the SCLC cell lines tested and in
5 of 10 tumors.   For comparison, Panel E shows CGH
analysis of chromosome 5 from NCI-H433.   This chromosome
shows no detectable copy number changes.
30   Figure 2 summarizes CGH analyses of increases
and decreases in DNA copy number in 18 SCLC cell lines,
including 8 classic (radiosensitive) and 10 variant
(radioresistant) lines. Common copy number changes (i.e.
those occurring in > 30% of cell lines) are shown in
35   Table 1.   The regions listed in Table 1 represent the
smallest area on each chromosome arm that is altered in
a large number of the cell lines. For example, DNA copy

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number increases occur on the q arm of chromosome 8 in
12/18 cell lines. However, the extent of the altered
region is somewhat different among the cell lines (Figure
2). The region of most common abnormality is listed as

5   8q24 because this region, which includes the most distal
3 Giemsa bands (8q24.1, 8q24.2, and 8q24.3 respectively)
on 8q, is the minimal region of overlap among cell lines
showing a DNA copy number increase in this area.
Centromeric regions have not been included in the

10   summaries in Figure 2 and Table 1 because CGH analyses
are not informative in these regions (hybridization is
almost completely inhibited in these regions by the
excess   unlabeled   Cot-1   DNA   included   during
hybridization). We have confirmed that most of the

15   alterations identified here in cell lines also occur in
small cell lung tumors. The abnormalities found
frequently in the cell lines that have been identified in
tumors to date are indicated by a "+" in the "Tumor"
column in Table 1.

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DNA copy number alterations

       DNA copy number changes detected in 18 SCLC
cell lines are summarized in Figure 2 and Table 1. Some
of these occur at regions previously reported to be

25   aberrant in SCLC. For example, copy increases are seen
in 10 of 18 cell lines at 1p22-32, the site of L-myc, and
in 7 of 18 cell lines at 2p24-25, the site of N-myc. We
also observed copy number increases in 9 of 18 cell lines
at 6q22 and in 12 of 18 cell lines at 8q24, the sites of

30   the c-myb and c-myc oncogenes, respectively. These
oncogenes have been shown to be amplified in many SCLC
tumors and cell lines (Little et al. (1983), *Nature* 306,
194-196; Griffin et al. (1985) *Cancer Research* 45,
272-275). We observed copy number decreases on 3p in 15

35   of 18 cell lines, a genetic alteration that is frequently
observed cytogenetically in SCLC (Whang-Peng et al.
(1982), *Cancer Genetics and Cytogenetics* 6, 119-134). No

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tumor suppressor gene has yet been identified on 3p, but LOH studies suggest that there are at least two and possibly three such tumor suppressor genes on this chromosome arm (Carbone and Minna, 1992). We also
5   observed copy number decreases in 7 of 18 cell lines at 13q, and in 12 of 18 cell lines on 17p, the sites of the RB and p53 tumor suppressor genes, respectively. Both of these tumor suppressor have been previously shown to be frequently altered in SCLC (Harbour et al. (1988) *Science*
10 241, 353-357; Takahashi et al. (1989), *Science* 246, 491-494).

CGH analysis revealed several new sites of frequent DNA copy number increase or decrease. Among the most common sites of copy number increases are 1q24 (11
15 of 18 cell lines), 5p (11 of 18 cell lines) and Xq26 (10 of 18 cell lines). Many of the amplifications in the 1q24 region overlap with a novel region of amplification at 1q32 commonly found in breast tumors (Kallioniemi et al., (in press.) "Detection and mapping of amplified DNA
20 sequences in breast cancer by comparative genomic hybridization" *Proc. Natl. Acad. Sci. USA*). The copy number increases that occur on chromosome 5p are unusual in that they are frequently high-level increases (in 6 of 11 cases), despite the fact that they apparently involve
25 the entire chromosome arm. Frequent DNA copy number increases on 5p have also been observed in non-SCLC tumors. Other DNA copy number increases were detected at 1p22-32, 2p24-25, 3q22-25, 13q3, 18p11.1-11.2 and 18q21. Newly identified regions of copy number decrease in SCLC
30 include 22q12.1-13.1, 10q26, 16p11.2 and 19p13.3, which are all affected in at least 13 of the 18 cell lines (Figure 2 and Table 1). Other DNA copy number decreases were detected at 18p and 10p.

Table 1: Minimal Overlapping Regions of Common Copy Number Change in SCLC

	Chromosomal position	Classic	Frequency Variant	Total	Known Gene	Tumor
Copy Number Increases:	8q24.1	6/8	6/10	12/18	c-myc (8q24)	+
	5p	4/8	7/10	11/18		+
	1q24	4/8	7/10	11/18		+
	Xq26	4/8	6/10	10/18		+
	1p22-32	2/8	8/10	10/18	L-myc (1p32)	+
	Xp22.1	3/8	6/10	9/18		
	6q22	4/8	5/10	9/18	c-myb (6q22)	+
	20p12-13	6/8	3/10	9/18		
	11q14-22.2	3/8	6/10	9/18		
	7p21	3/8	5/10	8/18		
	7q31.2-31.3	3/8	5/10	8/18		
	14q12-13	2/8	6/10	8/18		
	14q31	2/8	6/10	8/18		
	2p24-25	1/8	6/10	7/18	N-myc (2p24)	
	3q22-25	1/8	6/10	7/18		+
	18q21	6/8	1/10	7/18		+
Copy Number Decreases:	22q12.1-13.1	6/8	10/10	16/18		+
	3p13-14	8/8	7/10	15/18	*	+
	3p21.3	8/8	6/10	14/18	*	+
	10q26	6/8	7/10	13/18		+
	16p11.2	4/8	9/10	13/18		+
	19p13.3	6/8	7/10	13/18		+
	17p	5/8	7/10	12/18	p53 (17p13)	+
	16q11.22	7/8	4/10	11/18		+
	4q24-26	5/8	5/10	10/18		+
	15q11-14	6/8	4/10	10/18		+
	19q13.3-13.4	3/8	7/10	10/18		+
	10p	7/8	2/10	9/18		+
	13q11-13	3/8	4/10	7/18	RB (13q14)	+

Note: In Table 1, chromosomal copy number changes occurring in greater than 30% (6/18) cell lines are listed, with the relative distribution in classic and

20

variant cell lines indicated. The chromosomal positions of the copy number changes were assigned based on comparison of the two-color (red and green) comparative genomic hybridization images with the DAPI banding image and based on quantification of green to red ratios along each chromosome axis. The frequency of each copy number change in classic and variant cell lines is based on the data presented in Figure 2. The oncogenes and tumor suppressor genes listed in the "Known Gene" column are those previously shown to be affected in SCLC cell lines and/or tumors. The "*" symbol in the rows for 3p copy number decreases refers to several as yet unidentified tumor suppressor genes in this region. A "+" symbol in the "Tumor" column indicates that this alteration is observed in at least 30% (3/10) of the SCLC tumors analyzed so far.

Example 2: Correlation of genomic amplifications and deletions with radioresistance

An important reason for analyzing the genetic alterations in SCLC cell lines was to identify specific changes that correlate with phenotypes of these cell lines, including their resistance to ionizing radiation. Over sixty percent of all cancer patients are treated with ionizing radiation during the course of therapy and yet no reliable predictive assays for tumor radioresponsiveness are currently available (Weichselbaum (1991) *Important Advances in Oncology* 73-83) The radioresponsiveness of tumors *in vivo* varies considerably within one histological type such as SCLC. *In vivo* radioresponsiveness is a complex phenomenon, involving factors such as the degree to which the tumor is hypoxic (and, therefore, radioresistant), the kinetics of tumor growth, and the level of tumor differentiation. Despite these complications, the failure of radiotherapy to achieve local control of tumor growth is correlated with radioresistance in cell lines derived from both soft

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tissue sarcomas and head and neck carcinomas
(Weichselbaum et al. (1990), *International Journal of Radiation Oncology, Biology, Physics*, 19(2), 313-319).
This suggests that tumor radioresistance arising from
5   phenotypic changes caused by genetic aberrations may be
an important reason for radiotherapy failure
(Weichselbaum, 1991, Suit (1986) *Int. J. Radiat. Oncol. Biol. Phys.*, 12, 453-458). Localization of DNA copy
number aberrations that correlate with radioresponse
10   allows for a simple predictive assay for *in vivo* tumor
radioresistance and may lead to the discovery of specific
genes which correlate to radioresistance, as well as the
development of new procedures for overcoming
radioresistance.

15   Using the methods described above in Example 1,
We have identified several DNA copy number changes that
correlate well with the "variant" SCLC phenotype (Table
2), which is associated with increased radioresistance
(Carmichael et al. (1989) *Eur. J. Cancer Clin. Oncol.*
20   25, 527-534). Copy number gains on 1p22-32, 2p24-25 and
3q22-25 and losses 18p are preferentially seen in cell
lines of the variant phenotype (Chi-square p value <
0.1), whereas gains at 13q3, 18p11.1-11.2, and 18q21 and
losses at 10p are preferentially seen in cell lines of
25   the classic phenotype (Chi-square p value < 0.1). Some
of these loci may contain genes that play a role in
radioresistance or other aspects of the variant
phenotype. Since the variant phenotype includes a number
of morphological and biochemical characteristics, it is
30   possible that the genetic alterations leading to this
phenotype involve transcription factors or other
regulatory proteins whose overexpression switches the
tumor cell to an alterative developmental fate. For
instance, overexpression of the c-myc oncogene in classic
35   SCLC lines by DNA transfection has been shown to result
in a partial variant phenotype (Johnson et al. (1986),
Journal of Clinical Investigation 78, 525-532). Our

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results indicate that a good correlation exists between the variant phenotype and increased copy number at 1p22-32 (L-myc) and 2p24-25 (N-myc). The radioresistant phenotype of variant SCLC cell lines might be explained

5 by an increase in expression of DNA repair enzymes capable of repairing the double strand breaks caused by ionizing radiation or, perhaps, by a change in the cell cycle distribution, a parameter that is known to affect radioresistance (Hall (1988) *Radiobiology for the*

10 *Radiologist*, 3rd edition, J.B. Lippincott, Philadelphia, PA.). In addition to L-myc, the GADD45 gene, which was identified based on the fact that it is transcriptionally activated in response to ionizing radiation (Fornace et al. (1989), *Mol. Cell. Biol.* 9, 4196-4203) maps to the

15 site of variant-specific amplification observed in these studies. Finally, the regional amplification observed at 11q23 which occurred in 4 variant cell lines and no classic cell lines overlaps the Ataxia Telangiectasia locus (Gatti et al. (1988), *Nature* 336, 577-580).

20 Standard FISH probe technology will allow investigators to determine whether these genes are involved in the radioresistance phenotype of variant cell lines.

Table 2: Copy number changes that correlate with the variant or classic phenotype					
			Frequency		
Variant-specific	Chromosomal position	Classic lines	Variant lines	Total	Chi-Square P-value
Copy Number Increases	1p22-32	1/8	8/10	9/18	<0.05
	2p24-25	1/8	6/10	7/18	<0.1
	3q22-25	1/8	6/10	7/18	<0.1
Copy Number Decreases	18p	0/8	5/10	5/10	<0.025
Classic-specific					
Copy Number Increases	13q3	4/8	1/10	5/18	<0.1
	18p 11.1-11.2	4/8	1/10	5/18	<0.1
	18q21	5/8	1/10	6/18	<0.1
Copy Number Decreases	10p	6/8	2/10	8/18	<0.1

Example 3: Genetic aberrations in non-small cell lung cancers (N-SCLC) detected by CGH

In order to detect genetic aberrations in N-SCLCs, tumor DNAs were analyzed using CGH as per Example 1. DNAs from five N-SCLC cell lines (PC3, PC7, PC13: adenocarcinomas, PC10:Squamous cell carcinoma, and PC14: large cell carcinoma) and 20 primary lung cancers (11 adenocarcinomas, 6 squamous cell carcinomas, 1 small cell carcinoma, 1 small+ squamous cell combined type carcinoma, and 1 large cell carcinoma) were used in the CGH analysis to generate green/red intensity profiles of each chromosome analyzed. Chromosomal regions showing a green/red ratio greater than 1.45 were considered to be

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amplified, while a green/red ratio of less than 0.75 was considered to contain a deletion.

Figure 3 shows amplified and deleted chromosomal regions in 5 lung cancer cell lines. Figure
5 4 shows the amplified and deleted chromosomal regions in 20 primary lung cancers. In cell lines, the frequently amplified regions were 3q (4/5), 5p (3/5), 6p (3/5), 7q (3/5), 8q (3/5), 9q (3/5), and 20p (4/5) The frequently deleted regions were 3p (2/5), 4q (3/5), 6q (2/5), 9p
10 (2/5), 9q (2/5), 10p (3/5), 11q (2/5), 13q (3/5), 14q (3/5), 15q (2/5), 17p (3/5), 18q (3/5), 22q (2/5) and X (3/5). In the primary lung cancers which were examined, the frequently amplified regions were 3q (6/20), 5p (6/20), 7p (3/20) and 8q (5/20). In addition, one case
15 of 20q amplification that had been previously reported for breast cancer was observed in an adenocarcinoma. The frequently deleted regions in the primary tumors were 1p (11/20), 10q (4/20), 16p (8/20), 17p (9/20), 19p (14/20), 19q (15/20) and 22q (6/20). The regions which were found
20 to be amplified in both primary tumors and N-SCLC cell lines are 3q, 5p, 7p, 7q, 8q and 9q.

Some known oncogenes, for instance, MYC (C-myc) (8q24), EGFR (V-erb-b) (7p12), KRAS1P (6p12-p11) and NRASL3 (6pter-p21) are located in the commonly amplified
25 regions observed in this Example. Additional unknown oncogenes may be located in the chromosomal regions 3q, 5p and 7q. The loci of known tumor suppressor genes APC (5q21-22), WT1 (11p13), RB1 (13q14.2) TP53 (17p13.1 and DCC (18q21.1) are involved in the deleted region in cell
30 lines, but most of them are not involved in the primary tumors which were studied herein.

The gain and loss of chromosomal regions and the corresponding correlation with the survival rate of patients with primary non-small cell lung carcinomas are
35 shown in Tables 3 and 4.

Table 3: Chromosomal alterations in N-SCLC cell lines and primary tumors with corresponding mortality.

Name	Histology	P-stage	Chromosomal gain	Chromosomal Loss	Frequent Loss	Rec-currance	Survival
PC3	AD		1q, 2q, 6q, 15	8q, 9q, 11p, 13q, 14q, 18q	17p, 19q		
PC7	AD		1p, 1q, 3q, 4p, 4q, 6p, 7p, 7q, 8p, 8q, 9q, 12q, 13q, 15, 20p	3p, 4q, 6q, 11q, 18q	17p, 22q		
PC10	SQ		1q, 2p, 2q, 3q, 5p, 7p, 7q, 20p	1p, 1q, 4p, 4q, 5q, 8p, 9q, 10p, 13, Xp	19p		
PC13	AD		3q, 5p, 6p, 7q, 8q, 9q, 20p	2p, 3p, 4q, 6q, 10p, 11q, 14q, 18q, 21q, Xp, Xq	16p, 17p, 22q		
PC14	LA		1p, 2p, 2q, 3p, 3q, 5p, 6p, 9q, 10q, 14, 16q, 20p	9p, 10p, 21q	(-)		
NCI-H69	SM		1q, 3q, 6p, 6q, 7q, 9p, 11p, 13q, 18p, 18q	1p, 2p, 2q, 3p, 3q, 4p, 4q, 7p, 8q, 10p, 10q, 13q, 20q	17p		
M.H.	Fibro-sarcoma	IV	5q, 13q, 18q	16q	16p, 17p, 19p, 19q		
O.F.	Renal Cell Carcinoma	IV	9p	(-)	22p, 22q		

Table 3: Chromosomal alterations in N-SCLC cell lines and primary tumors with corresponding mortality.
(Continued from the previous page)

	Neurinoma	Benign					
M.T.	Neurinoma	Benign	(-)	(-)	22q	(+) 31 M	Alive (4B)
Y.T.	AD	IIIA	Xp, Xq	9q, 10q	1p, 19p, 19q	(-) renal failure (af)	Dead (1M)
M.T.	AD	II	5p	1p	22q	(-) pneumonia	Dead (2M)
M.T.	AD	IV (pm)	15q	2q, 10q, 14q	17p	(-)	Alive (50M)
Y.A.	AD	IIIA	3q	(-)	1p, 16p, 19p, 19q	(+)	Dead (4M)
K.H.	AD	IIIB	5p, 7p, 14q, 20q	2q	16p, 17p, 19p, 19q, 22q	(+)	Dead (14M)
K.K.	AD	IIIA	3q, 5q, 8q, 15q	8p, 9q, 10p, 10q, 14q	17p, 19p, 22q	(+)	Dead (31M)
Y.K.	AD	IIIA	(-)	(-)	1p, 19p, 19q, 22q	(-)	Alive (45M)
K.K.	AD	I	2q	(-)	1p, 16p, 19p, 19q	(-)	Alive (50M)
G.T.	AD	II	5p, 5q, 7p, 8q	8p	19p, 19q	(+)	Dead (6M)
Y.S.	AD	IIIA	(-)	(-)	19p, 19q		

Table 3: Chromosomal alterations in N-SCLC cell lines and primary tumors with corresponding mortality.
(Continued from the previous page)

T.M.	AD	IIIA	1q, 6p, 15q, 21q	(-)	19p, 19q	(?)	Alive (30M)
K.Y.	SQ	IIIA	3q	(-)	1p	(+)	Dead (40M)
K.H.	SQ	IIIB	3q, 9p	9q, 12q	1p, 17p, 19p, 19q	(+) 36 M	Dead (40M)
H.K.	SQ	IIIA	1q, 5p, 7p, 8q	(-)	1p, 17p, 19p, 19q, 22q	(?)	Alive (3M)
H.I.	SQ	IV	(-)	(-)	1p, 17p, 19q, 22q	(+)	Dead (24M)
S.K.	SQ	IIIA	3q, 5p, 6p, 6q, 7q, 8q, 12p, 12q, Xp, Xq	(-)	1p, 16p, 16q, 17p	(+)	Dead (4M)
S.I.	SQ	IIIA	(-)	(-)	1p, 16p, 19p, 19q	(-) MRSA	Dead (3M)
H.T.	SM	I	5p, 8q	4q, 5q, 10p, 10q, 16q	16p, 17p	(-)	Alive (23M)
M.H.	SM+SQ	IIIA	3q	3p, 5q, 16q	16p, 17p, 19p, 19q, 22q	(-) sepsis	Dead (1M)
S.T.	LA	I	(-)	(-)	1p, 16p, 19p, 19q,	(-)	Alive (33M)

Ad= Adenocarcinoma, SQ= squamous cell carcinoma, SM= small cell carcinoma, LA= large cell carcinoma
Deletion= excludes region around centromeres.

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Table 4: Correlation of survival rate to gain or loss of chromosomal region.

Chrom-osomal gain/ no gain	Chrom-osomal Loss/ no loss	Number	Wilcoxon N.S.	Cox-Mantel N.S.	1 year survival rate/ 3 year survival rate	Median Survival Term (months)
3q gain		6	.04132	.782009	80%/ 60%	14
3q no gain		14			80%/ 54.9%	23
5p gain		6	.4545	.5726	50%/ 50%	4
5p no gain		14			90.9%/ 62.3%	30
8q gain		5	.1749	.3987	75%/ 50%	4
8q no gain		15			81.8%/ 62.3%	30
	1p loss	11	1.2559	.6971	88.9%/ 66.7%	33
	1p no loss	9			66.7%/ 50%	4
	16p loss	8	.6570	.6037	66.7%/ 66.7%	4
	16p no loss	12			88.9%/ 53.3%	24
	17p loss	9	1.522	2.4759	66.7%/ 25.0%	4
	17p no loss	11			88.9%/ 76.2%	33
	22q loss	7	$p < .05$ (2.1833)	$p < .01$ (2.664)	75% 0%	4
	22q no loss	13			81.8%/ 81.8%	30

Note: the relationship between pathological stage and survival rate is as follows: p-stage I+II (n = 5) 1 year survival rate = 100%, 3 year survival rate = 100% median survival term = 23 months; p-stage III+IV (n=15) 1 year survival rate = 75%, 3 year survival rate = 48.6%, median survival rate = 14 months (Wilcoxon N.S. = 0.3061; Cox-Mantel (p < .05) = 1.990).

The relationship between the number of chromosomal gains and the survival rate in this study is as follows: gains ≤ 3 (n = 14) 1 year survival rate = 90%, 3 year survival rate = 67.5%, median survival term = 24 months. For gains ≥ 4 (n = 6) the 1 year survival rate = 60%, 3 year survival rate = 40%, median survival term = 4 months.

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The above examples are provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference.

30

WHAT IS CLAIMED IS:

1.   A   method   of   detecting   a   chromosome
abnormality   correlated   with   lung   cancer,   the   method
comprising:

5   contacting a nucleic acid sample from a patient
with   a   probe   which   binds   selectively   to   a   target
polynucleotide   sequence   correlated   with   lung   cancer,
wherein   the   probe   is   contacted   with   the   sample   under
conditions   in   which   the   probe   binds   selectively   with   the
10   target polynucleotide sequence to form a hybridization
complex; and
            detecting   the   formation   of   a   hybridization
complex.

15   2.   The   method   of   claim   1,   wherein   the
chromosome abnormality is a deletion.

3.   The   method   of   claim   1,   wherein   the
chromosome abnormality is an amplification.
20
4.   The method of claim 1, wherein the nucleic
acid sample is from a sputum sample from the patient.

5.   The method of claim 1, wherein the lung
25   cancer is a small cell lung cancer.

6.   The method of claim 1, wherein the lung
cancer is a non-small cell lung cancer.

30   7.   The method of claim 1, wherein the probe
is labeled with digoxigenin or biotin.

8.   The method of claim 1, wherein the step of
detecting the hybridization complex is carried out by
35   detecting a fluorescent label.

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9.   The method of claim 8, wherein the
fluorescent label is FITC.

10.   The method of claim 1, wherein the sample
5   comprises a metaphase cell.

11.   The method of claim 1, wherein the target
polynuclotide sequence is on chromosomal region selected
from the group consisting of 1q24, 5p, Xq26, 22q12.1-
10   13.1, 10q26, 16p11.2, and 19p13.3.

12.   The method of claim 1, wherein the target
polynuclotide sequence is on chromosomal region selected
from the group consisting of 3q, 5p, 8q, 1p, 16p, 17p,
15   22q.

13.   A kit for the detection of a chromosome
abnormality correlated with lung cancer, the kit
comprising a compartment which contains a nucleic acid
20   probe which binds selectively to a target polynucleotide
sequence in a region of a chromosome correlated with lung
cancer.

14.   The kit of claim 13, wherein the probe is
25 · labeled.

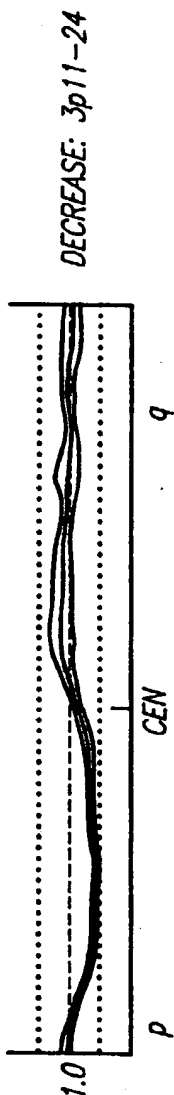15.   The kit of claim 14, wherein label is
selected from the group consisting of digoxigenin and
biotin.
30
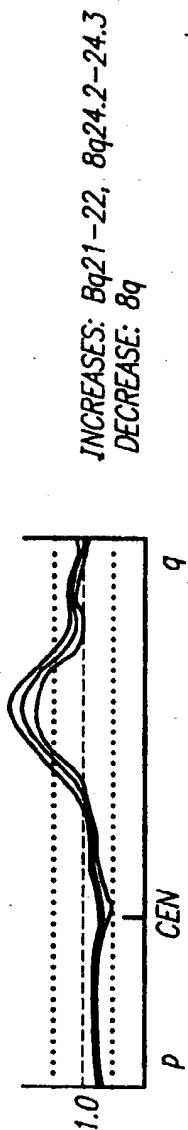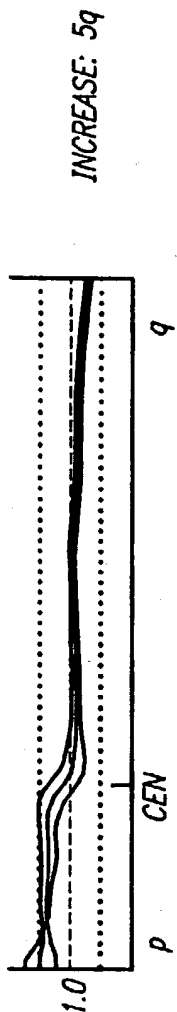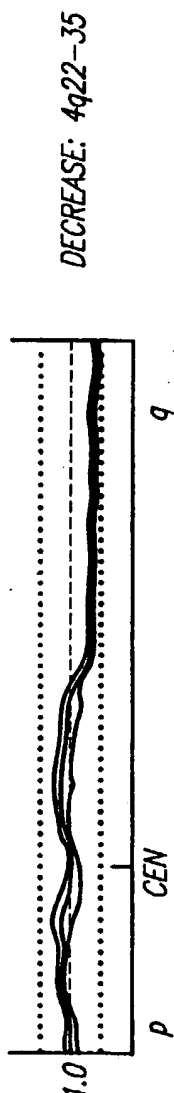
FIG. 1A

FIG. 1B

FIG. 1C

FIG. 1D

FIG. 1E
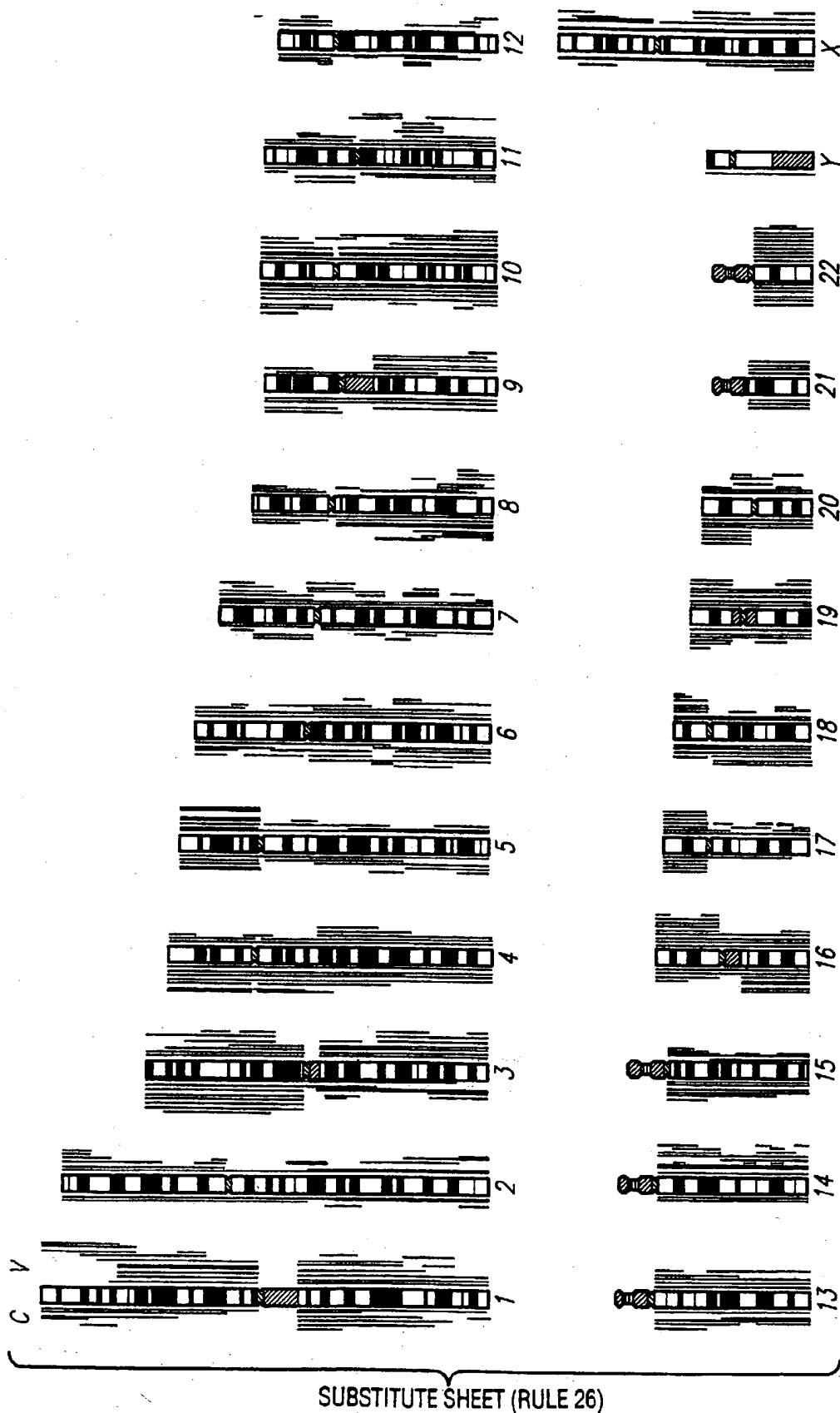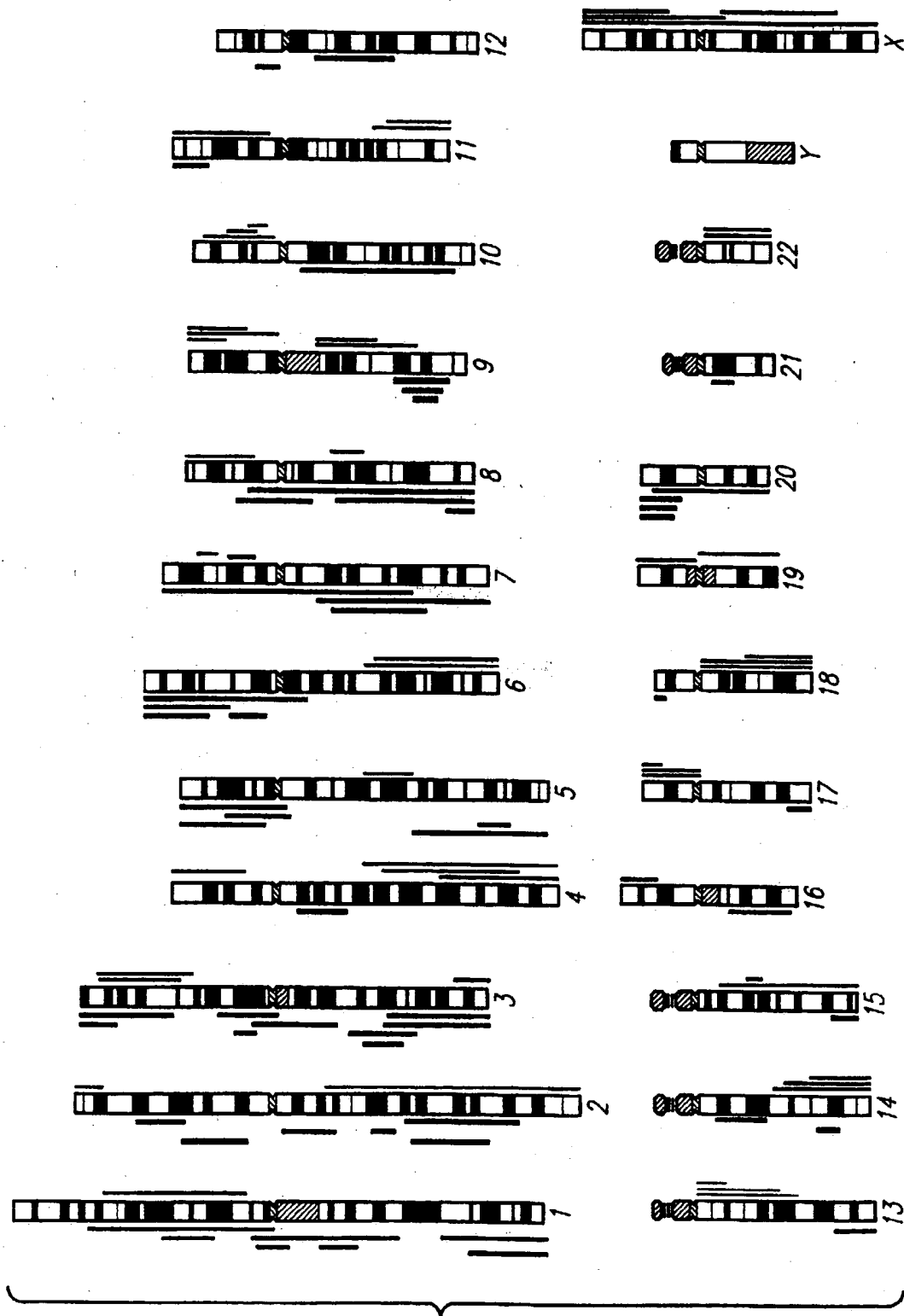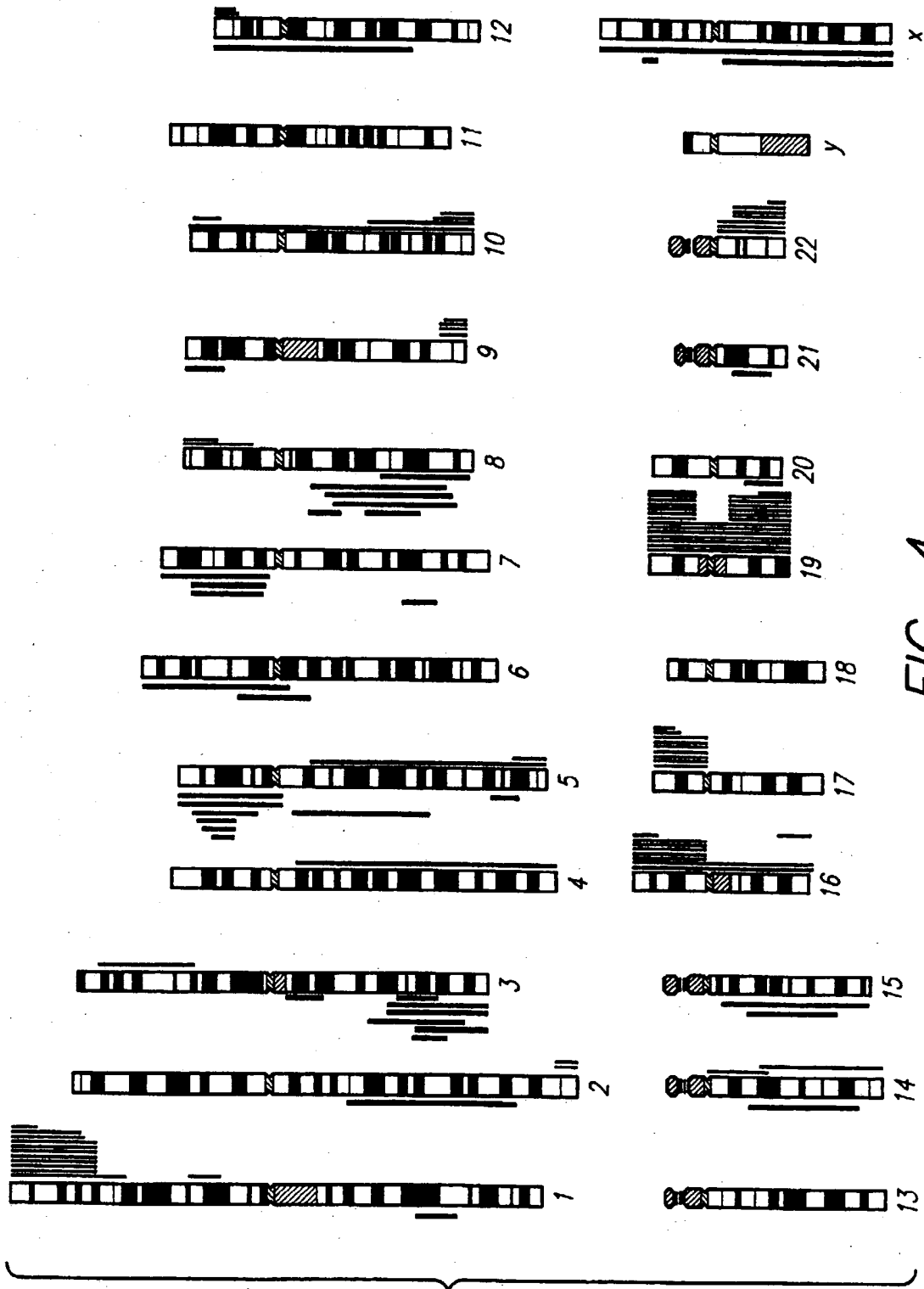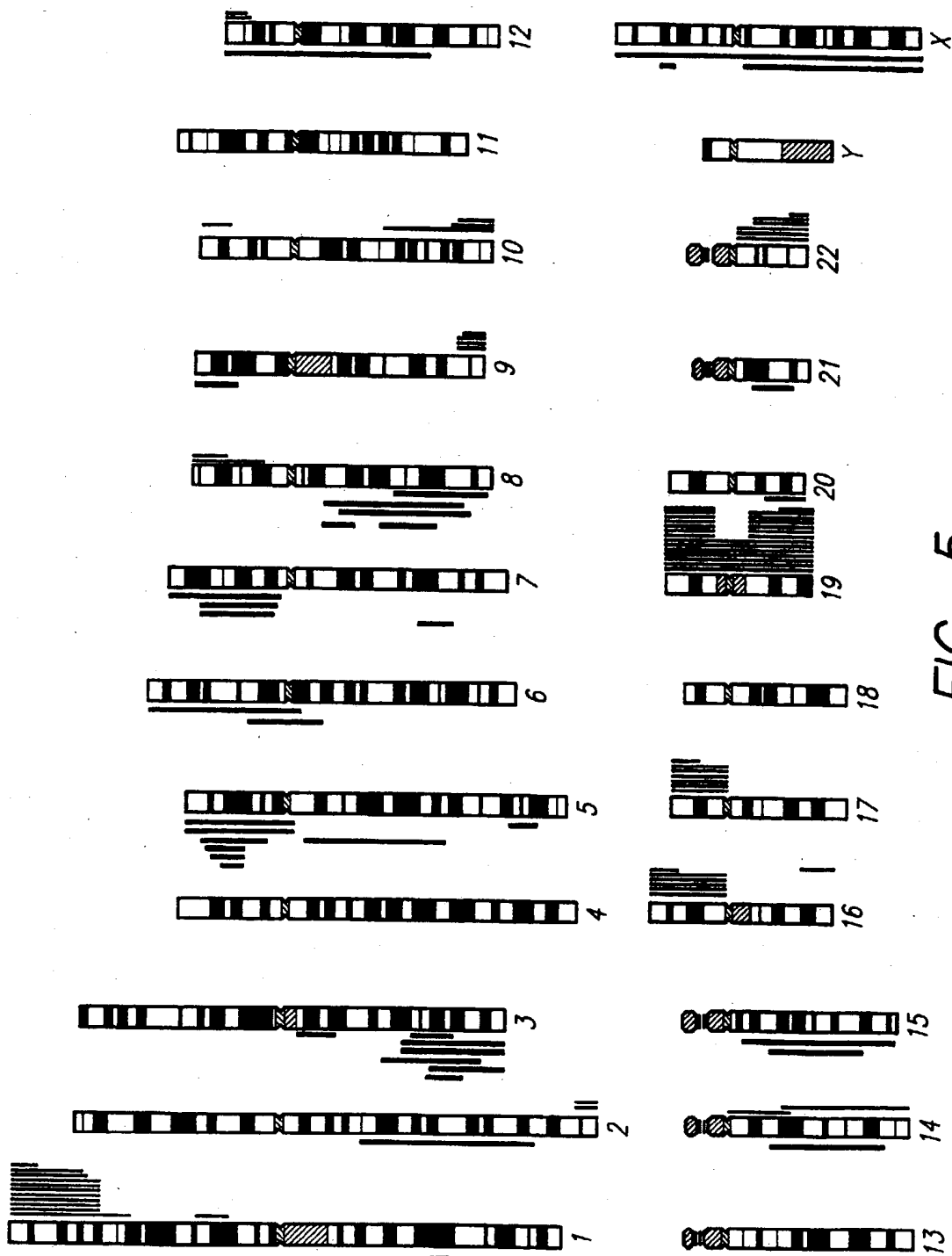
FIG. 2

FIG. 3

4/5

FIG. 4

FIG. 5

INTERNATIONAL SEARCH REPORT

A.	CLASSIFICATION OF SUBJECT MATTER

IPC(6)   :C12Q 1/68   -
US CL   :435/6

According to International Patent Classification (IPC) or to both national classification and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :   435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS, APSM, BIOSIS

C.	DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CANCER, VOLUME 60(11), ISSUED 01 DECEMBER 1987, CLINE ET AL, "ABNORMALITIES OF PROTOONCOGENES IN NON-SMALL CELL LUNG CANCER", (BIOSIS ABSTRACT), SEE ENTIRE DOCUMENT.	1-10
Y	HUMAN MUTATION, VOLUME 2(2), ISSUED 1993, TAKEDA ET AL, "DETECTION OF K-RAS MUTATION IN SPUTUM BY MUTANT-ALLELE-SPECIFIC AMPLIFICATION", (BIOSIS ABSTRACT). SEE ENTIRE ABSTRACT.	1-15
Y	SCIENCE, VOLUME 246, ISSUED 27 OCTOBER 1989, TAKAHASHI ET AL, "P53: A FREQUENT TARGET FOR GENETIC ABNORMALITIES IN LUNG CANCER", PAGES 491-494, SEE ENTIRE DOCUMENT.	1-15

X	Further documents are listed in the continuation of Box C.		See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 MARCH 1995	**30 MAR 1995**

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INTERNATIONAL SEARCH REPORT

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NEW ENGLAND JOURNAL OF MEDICINE, VOLUME 317, NUMBER 18, ISSUED 1987, BRAUCH ET AL, "MOLECULAR ANALYSIS OF THE SHORT ARM OF CHROMOSOME 3 IN SMALL-CELL AND NON-SMALL-CELL CARCINOMA OF THE LUNG", PAGES 1109-1113, SEE ENTIRE DOCUMENT.	1-15
Y	CANCER RESEARCH, VOLUME 52, ISSUED 01 MARCH 1992, MIURA ET AL, "CHROMOSOME ALTERATIONS IN HUMAN SMALL CELL LUNG CANCER: FREQUENT INVOLVEMENT OF 5Q1", PAGES 1322-1328, SEE ENTIRE DOCUMENT.	1-15
Y	NATURE, VOLUME 306, ISSUED 10 NOVEMBER 1983, LITTLE ET AL, "AMPLIFICATION AND EXPRESSION OF THE C-MYC ONCOGENE IN HUMAN LUNG CANCER CELL LINES", PAGES 194-196, SEE ENTIRE DOCUMENT.	1-15
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